WE CLAIM:

1. A microfluidic device for separating the components of a fluid sample, the microfluidic device comprising:

a substrate having a microchannel formed in a surface thereof;

a cover plate arranged over the substrate surface, the cover plate in combination with the microchannel defining a separation conduit for separating the components of the fluid sample according to a specific component property, wherein the separation conduit has an inlet port and an outlet port; and

an integrated gradient-generation means for generating a gradient of a selected mobile-phase component in a mobile phase, and adopted to allow the mobile phase from the gradient-generation means to be transported through the inlet port into the separation conduit and out of the outlet port.

2. The microfluidic device of claim 1, wherein the integrated gradient-generation means is formed at least in part within the substrate.

3. The microfluidic device of claim 1, wherein the integrated gradient-generation means is formed at least in part in the cover plate.

4. The microfluidic device of claim 1, wherein the integrated gradient-generation means comprises:

a mobile-phase holding conduit having a length defined by an upstream terminus and a downstream terminus;

a plurality of mobile-phase inlet ports arranged along the length of the mobile-phase holding conduit;

a mobile-phase outlet port located downstream from the mobile-phase inlet ports; and

a means for introducing the mobile phase from the mobile-phase holding conduit through the mobile-phase outlet port and into the inlet port of the separation conduit.

5. The microfluidic device of claim 4, wherein the mobile-phase inlet ports are evenly spaced along the length of the mobile-phase holding conduit.

6. The microfluidic device of claim 4, wherein no mobile-phase inlet port is located at the upstream terminus of the mobile-phase holding conduit.

7. The microfluidic device of claim 4, wherein no mobile-phase inlet port is located at the downstream terminus of the mobile-phase holding conduit.

8. The microfluidic device of claim 4, wherein the gradient-generation means further comprises a distribution conduit in fluid communication with the mobile-phase holding means.

9. The microfluidic device of claim 8, wherein the distribution conduit is substantially parallel to the mobile-phase holding conduit.

10. The microfluidic device of claim 8, wherein the distribution conduit further comprises a plurality of outlet ports, each fluidly communicating via a mixing conduit with an inlet port of the mobile-phase conduit.

11. The microfluidic device of claim 10, wherein the distribution conduit further comprises an inlet port located between each of its outlet ports.

12. The microfluidic device of claim 8, wherein the distribution conduit comprises two inlet ports.

13. The microfluidic device of claim 12, further comprising two mobile-phase sources in fluid communication with the integrated gradient-generation means, wherein at least one of the mobile-phase sources contains the selected mobile-phase component.

14. The microfluidic device of claim 13, wherein one of the mobile-phase sources does not contain the selected mobile-phase component.

15. The microfluidic device of claim 13, wherein one of the mobile-phase sources contains the selected mobile-phase component in a substantially pure form.

16. The microfluidic device of claim 1, further comprising a means for controlling fluid communication between the gradient-generation means and the separation conduit.

17. The microfluidic device of claim 1, further comprising separation media within the separation conduit.

18. The microfluidic device of claim 1, further comprising a polymeric material formed *in situ* within the separation conduit.

19. The microfluidic device of claim 1, wherein the separation conduit exhibits a high surface area-to-volume ratio.

20. The microfluidic device of claim 1, wherein the component property is selected from the group consisting of molecular weight, polarity, hydrophobicity, and charge.

21. A method for separating the components of a fluid sample, comprising:

(a) providing the microfluidic device of claim 1;

(b) using the integrated gradient-generation means to generate a gradient of the selected mobile-phase component in the mobile phase,

(c) controllably introducing a predetermined volume of the fluid sample from a sample source into the separation conduit; and

(d) conveying the fluid sample through the separation conduit using a mobile phase, thereby separating the components of the fluid sample.

22. The method of claim 21, further comprising, during or after step (d), (e) analyzing the fluid sample flowing in the separation conduit or from the outlet port of the separation conduit.

23. The method of claim 21, wherein step (b) further comprises (b') transporting components of the mobile phase into the integrated gradient-generation means.

24. The method of claim 23, wherein step (b) further comprises, after step (b'), allowing a sufficient amount of time to pass to result in diffusion of the components of the mobile phase to form a non-stepwise gradient in the mobile phase.

25. The method of claim 21, wherein step (d) is carried out using mobile phase flow at a rate of no more than about 1μL/min.

26. A microfluidic device for separating the components of a fluid sample, the microfluidic device comprising:

(a) a gradient-generation means for generating a gradient of a selected mobile-phase component in a mobile phase comprising

(i) a substrate having a microchannel formed in a surface thereof, wherein the microchannel has an upstream terminus and a downstream terminus,

(ii) a cover plate arranged over the substrate surface, wherein the cover plate, in combination with the microchannel, forms a mobile-phase holding conduit having a length defined by the upstream terminus and the downstream terminus,

(iii) a plurality of inlet ports arranged along the length of the mobile-phase holding conduit, and

(iv) an outlet port located downstream from the inlet ports of the mobile-phase holding conduit;

(b) a separation conduit for separating the components of a fluid sample according to a specific component property; and

(c) a means for introducing the mobile phase from the gradient-generation means into the separation conduit.

27. A microfluidic device for separating the components of a fluid sample, the microfluidic device comprising:

a substrate having a microchannel formed in a surface thereof;

a cover plate arranged over the substrate surface, such that the cover plate, in combination with the microchannel, defines a separation conduit for separating the components of the fluid sample according to a specific component property, wherein the separation conduit has an inlet port and an outlet port; and

an integrated mobile-phase source comprising a microconduit having a length defined by an upstream terminus and a downstream terminus, the microconduit containing a mobile phase that exhibits a gradient of a selected mobile-phase component along the length of the microconduit,

wherein the integrated mobile-phase source is arranged to allow the mobile phase to be transported through the inlet port into the separation conduit and out of the outlet port.

28. A method for separating the components of a fluid sample, comprising:

(a) using no more than about 100 μL of fluid to produce a mobile phase containing a gradient of the selected mobile-phase component; and

(b) conveying a fluid sample through the separation conduit using the mobile phase containing a gradient of the selected mobile-phase component, thereby separating the components of the fluid sample.

29. A microfluidic device for producing a flow of mobile phase:

(a) mobile-phase source comprising

(i) a mobile-phase holding microconduit having a length defined by an upstream terminus and a downstream terminus, and an outlet port located at the downstream terminus, and

(ii) a mobile phase, contained in the mobile-phase holding microconduit, that exhibits differing concentrations of selected mobile-phase component along the length of the mobile-phase holding microconduit; and

(b) a means for pressurizing the microconduit to force the mobile phase within the microconduit to flow toward the downstream terminus along the length of the microconduit and out the outlet port.

30. The microfluidic device of claim 29, further comprising a separation conduit in fluid communication with the outlet port of the microconduit.

31. The microfluidic device of claim 29, wherein the microconduit is further defined by a substrate having a microchannel formed in a surface thereof in combination with a cover plate arranged over the substrate surface.

32. A method for producing a flow of mobile phase, comprising:

(a) providing a mobile-phase source comprising

(i) a mobile-phase-holding microconduit having a length defined by an upstream terminus and a downstream terminus, and an outlet port located at the downstream terminus, and

(ii) a mobile phase, contained in the mobile-phase holding microconduit, that exhibits differing concentrations of selected mobile-phase component along the length of the microconduit a substrate; and

(b) pressurizing the microconduit to force the mobile phase within the mobile-phase holding microconduit to flow toward the downstream terminus along the length of the microconduit and out of the outlet port.

33. The method of claim 32, wherein the mobile phase is introduced into a separation conduit after flowing out of the outlet port.

34. The method of claim 32, wherein the mobile-phase holding microconduit is further defined by a substrate having a microchannel formed in a surface thereof in combination with a cover plate arranged over the substrate surface.

35. The method of claim 32, wherein step (a) comprises:

(a') providing at least one inlet port in fluid communication with the mobile-phase holding microconduit, wherein the outlet port is located downstream from the at least one inlet port of the mobile-phase holding microconduit;

(a'') providing a plurality of mobile-phase sources each containing a mobile phase, wherein each mobile phase contains a different concentration of a selected mobile-phase component; and

(a''') introducing plugs of mobile phase from the mobile-phase sources through the at least one inlet port into the mobile-phase holding microconduit such that the plugs are arranged in a predetermined order along the length of the mobile-phase holding conduit, the predetermined order.

36. A microfluidic device for producing a flow of mobile phase, the microfluidic device comprising:

(a) a means for producing different concentrations of a selected mobile-phase component in different locations within a mobile phase comprising

(i) a mobile-phase-holding microconduit having a length defined by an upstream terminus and a downstream terminus,

(ii) an outlet port located at the downstream terminus of the mobile-phase holding microconduit, and

(iii) at least one inlet port in fluid communication with the mobile-phase holding microconduit upstream from the outlet port;

(b) a plurality of mobile-phase sources each containing a mobile phase, wherein each mobile phase contains a different concentration of a selected mobile-phase component;

(c) a means for introducing plugs of mobile phase from the mobile-phase sources through the at least one inlet port into the mobile-phase holding conduit such that the plugs are arranged in a predetermined order along the length of the mobile-phase holding conduit; and

(d) a means for pressurizing the microconduit to force the mobile phase within the microconduit to flow toward the downstream terminus along the length of the microconduit and out the single outlet port.

37. The microfluidic device of claim 36, further comprising a separation conduit in fluid communication with the outlet port of the microconduit.

38. The microfluidic device of claim 36, wherein the mobile-phase holding microconduit is further defined by a substrate having a microchannel formed in a surface thereof in combination with a cover plate arranged over the substrate surface.